SENSITIVITY OF THE FLUORESCENCE TEST FOR AMINO ACIDS*

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The fluorescence test, when used for the detection of amino acids, is superior to other tests in that the chromatograms obtained can be used both for elution of unchanged amino acids from the filter paper, and for their identification by means of other colour reactions, whether general or specific. The general view is that amino acids fluoresce only on filter paper or on other cellulose materials, after being heated to 100–120°; according to most authors, the sensitivity of this test is much lower than that of the ninhydrin test.

The purpose of the present investigations was:

(a) to determine the sensitivity of the ordinary and sensitized u.v. tests for more than twenty amino acids;

(b) to study the influence of the temperature and heating time on the intensity of the fluorescence;

(c) to compare the sensitivity of the test before and after the development of the chromatogram in the systems phenol–water and propanol–water;

(d) to adapt the u.v. test, as has been done with the ninhydrin test, to the detection of amino acids in biological fluids.

EXPERIMENTAL

1. *Materials and apparatus*

Standard amino acids of a high degree of purity.

Samples of physiological urines and sera.

Reagents sensitizing the u.v. test:

(a) 0.01 % xylose solution in ethanol used for soaking the filter paper.

(b) 0.01 % solution of sodium 1,2-naphthoquinone-4-sulphonate (NQS) in methanol used for soaking the filter paper.

U.v. lamp "Dedectolit" (filterglass OX1) emitting principally in the region 3500 Å.

U.v. lamp "Chromatolit" (filterglass OX7) emitting in the region 2537 Å.

2. *Procedure*

The term "specific sensitivity" of the test was applied to the smallest amount of a given amino acid (in μg) that produces a distinct fluorescence of the whole surface of

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the spot under strictly defined, standard experimental conditions. The standard conditions for the u.v. test are:

Filter paper: Whatman No. 1; volume of solution applied to the filter paper: 2.6 μl; area of the spot: 50.24 mm^2.

Developing solvents: phenol–water (7:3), propanol–water (7:3).

In order to determine the sensitivity after the development of the chromatogram, the area of the spot was measured with a planimeter and reduced to the starting surface.

The sensitivity of the test was determined either on untreated filter paper (ordinary test) or on filter paper soaked with a solution of NQS (sensitized test).

The comparison of the sensitivity of the u.v. test and of the ninhydrin reaction for the individual amino acids was based on the data contained in the paper by OPIEŃSKA-BLAUTH and co-workers[3].

TABLE I

SPECIFIC SENSITIVITIES OF AMINO ACIDS IN THE NINHYDRIN AND ISATIN REACTIONS AND IN THE ORDINARY AND SENSITIZED U.V. TESTS

No.	*Amino acid*	S_n μg	S_{iz} μg	$S_{u.v.}$ μg	$S_{u.v.s}$ μg	K_1	K_2	K_3
1	Alanine	0.065	0.13	0.26	0.026	4.0	2.0	0.4
2	β-Alanine	n.d.	0.13	0.26	0.026	—	2.0	—
3	α-Amino-n-butyric acid	n.d.	1.04	0.26	n.d.	—	0.2	—
4	Arginine	0.13	0.78	0.26	0.026	2.0	0.3	0.2
5	Asparagine	0.26	1.04	0.32	0.26	1.2	0.3	1.0
6	Aspartic acid	0.065	0.78	0.32	0.026	5.0	0.4	0.4
7	Glutamine	0.13	0.26	0.32	0.052	2.4	1.2	0.4
8	Glutamic acid	0.03	0.13	0.32	0.026	10.6	2.4	0.86
9	Glycine	0.02	0.13	0.26	0.026	13.0	2.0	1.3
10	Histidine	0.13	0.26	0.32	0.026	2.4	1.2	0.2
11	Hydroxyproline	n.d.	n.d.	1.0	0.26	—	—	—
12	Isoleucine	0.065	0.26	0.32	0.13	5.0	1.2	0.2
13	Leucine	0.03	0.13	0.32	0.026	10.6	2.4	0.86
14	Lysine	0.065	0.26	0.13	0.13	2.0	0.5	0.2
15	Methionine	0.03	0.26	0.32	0.052	10.6	1.2	1.7
16	Norleucine	0.065	0.52	0.32	0.052	5.0	0.6	0.8
17	Norvaline	0.13	0.52	0.26	0.052	2.0	0.5	0.4
18	Ornithine	0.065	0.52	0.26	0.026	4.0	0.5	0.4
19	Phenylalanine	0.065	0.26	0.32	0.026	5.0	1.2	0.4
20	Proline	0.52	0.03	1.8	0.13	3.4	60.0	0.25
21	Serine	0.03	0.13	0.26	0.026	8.6	2.0	0.86
22	Taurine	0.52	1.04	0.26	0.052	0.5	0.2	0.1
23	Threonine	0.065	1.04	0.32	0.13	5.0	0.3	2.0
24	Tryptophan	0.065	0.26	0.032	0.026	0.5	0.12	0.4
25	Valine	0.03	0.13	0.32	0.026	10.6	2.4	0.86

S = Sensitivity of the ninhydrin reaction. n.d. = not determined.
S_{iz} = Sensitivity of the isatin reaction.
$S_{u.v.}$ = Sensitivity of the fluorescence test.
$S_{u.v.s}$ = Sensitivity of the sensitized fluorescence test.

$$K_1 = \frac{S_{u.v.}}{S_n}\ ; \qquad K_2 = \frac{S_{u.v.}}{S_{iz}}\ ; \qquad K_3 = \frac{S_{u.v.s}}{S_n}\ .$$

Paper chromatography of the amino acids in serum was carried out after precipitation of proteins, while urine was desalted with an ion-exchange resin. Two-dimensional chromatograms were developed in the systems phenol–water and propanol–water. The spots were located by means of the u.v. lamp "Chromatolit".

3. *Results*

(A) A series of experiments, each of which was repeated several times, made it possible to determine the specific sensitivities of amino acids with regard to the u.v. test, both ordinary and sensitized (Table I). The coefficients K_1, K_2 and K_3 serve to compare the sensitivities of the individual amino acids in the ordinary and sensitized u.v. test with the sensitivities obtained in the ninhydrin and isatin reactions.

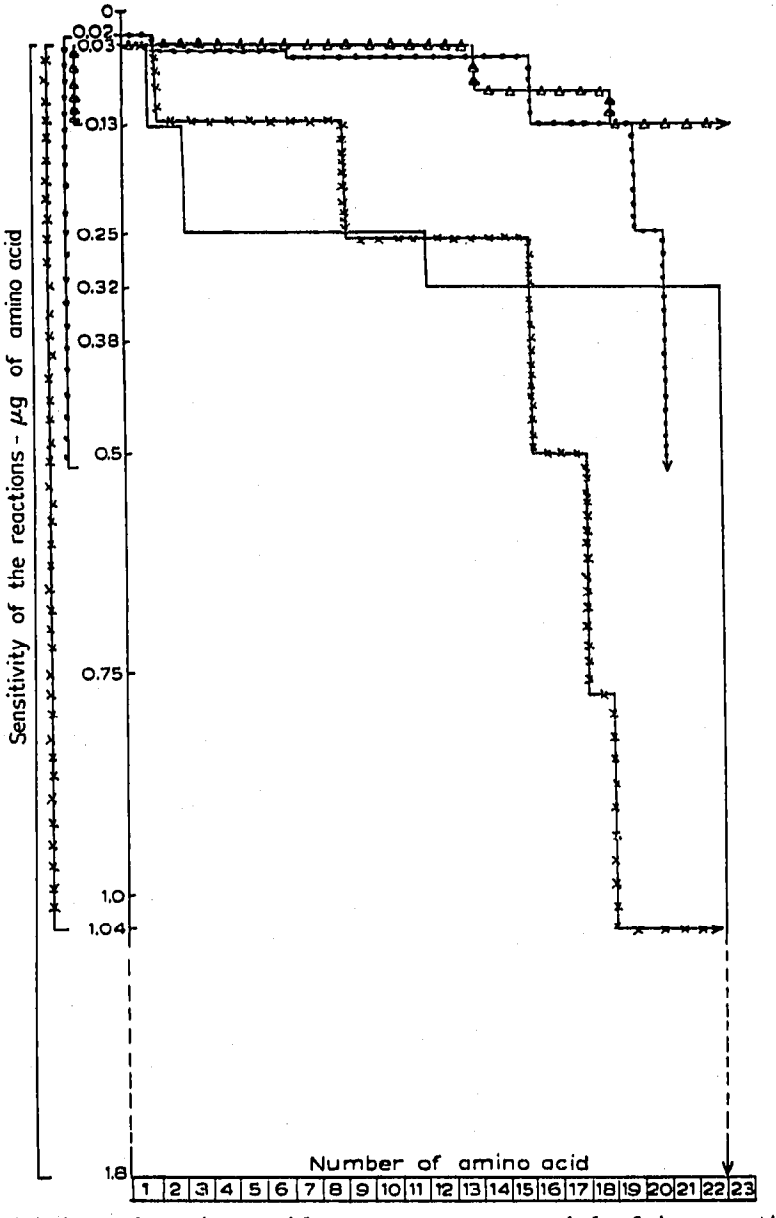

Fig. 1. Limits of sensitivity of amino acids. —•—•—•— ninhydrin reaction; —×—×—×— isatin reaction; ———— u.v. test; —△—△—△— sensitized u.v. test.

The sensitivity ranges of more than twenty amino acids in the ordinary and sensitized u.v. tests and in the ninhydrin and isatin reactions were also compared (Fig. 1).

(B) In order to determine the influence of various factors, such as temperature or time of heating of chromatograms before testing in u.v. light, comparative studies were carried out on amino acids not subjected to heating or heated to 70, 90, 110 and 120° for 2, 5 and 10 minutes (Tables II and III).

(C) The experiments resulted in the determination of the influence of the development of chromatograms in the systems phenol–water and propanol–water on the

TABLE II

EFFECT OF TEMPERATURE OF HEATING ON THE SENSITIVITY OF THE FLUORESCENCE TEST

	Amino acid	*μg*					
		20°	*40–50°*	*60–70°*	*80–90°*	*100–110°*	*110–120°*
1	Alanine	Lack of fluorescence	Fluorescence only on the periphery	0.65	0.52	0.52	0.26
2	β-Alanine			0.52	0.37	0.37	0.26
3	α-Amino-*n*-butyric acid			0.65	0.52	0.32	0.26
4	Arginine			0.52	0.32	0.26	—
5	Asparagine			n	0.65	0.52	0.32
6	Aspartic acid			n	n	n	0.32
7	Glutamine			n	0.65	0.65	0.32
8	Glutamic acid			n	0.65	0.65	0.32
9	Glycine			n	0.65	0.65	0.26
10	Histidine			n	n	0.32	0.32
11	Hydroxyproline			n	n	2.6	1.0
12	Isoleucine			n	n	n	0.32
13	Leucine			n	0.65	0.52	0.32
14	Lysine			0.52	0.32	0.26	0.13
15	Methionine			n	n	0.65	0.32
16	Norleucine			n	0.65	0.65	0.32
17	Norvaline			0.52	0.32	0.32	0.26
18	Ornithine			0.32	0.26	0.26	0.26
19	Phenylalanine			n	0.65	0.52	0.32
20	Proline			n	5.2	2.6	1.8
21	Serine			0.52	0.32	0.32	0.26
22	Taurine			n	0.52	0.32	0.26
23	Threonine			0.52	0.32	0.32	0.32
24	Tryptophan			n	n	0.52	0.032
25	Valine			0.65	0.52	0.52	0.32

n = lack of fluorescence.

sensitivity of the u.v. tests (Tables IV and V). The coefficient K expresses the relation of the sensitivity after development of the chromatogram to the specific sensitivity. In the calculations the ratio of the area of the spot after development to the standard area (50.24 mm^2) was taken into account.

(D) After desalting, samples of physiological urine were applied to the filter paper in various quantities, from 25 to 100 μl. Protein-free samples of sera were applied to the filter paper in 250 μl portions. Two-dimensional chromatograms were developed in the systems propanol–water and phenol–water. Amino acids were visualized by means of the u.v. tests and the ninhydrin reaction (Table VI).

DISCUSSION AND CONCLUSIONS

The importance of the fluorescence test for the detection of amino acids and its superiority to the ninhydrin reaction have been stressed by PHILLIPS[5] and WOIWOD[10,11] PHILLIPS gives the value of about 20 μg as the lower limit of the sensitivity of the u.v. test for most amino acids studied by him. Broadly speaking, most authors stress the low sensitivity of the u.v. reaction. According to WOIWOD, tryptophan, histidine, and citrulline are exceptions, their fluorescence sensitivity being much greater than that of other amino acids.

The phenomenon of the amino acid fluorescence in u.v. light is undoubtedly connected with cellulose. According to WOIWOD, the mechanism of the fluorescence is based on the reaction of the amino group of amino acids with the aldehyde group of saccharides[8]. The fluorescence appears only after heating. This is confirmed by WOIWOD's experiments with cellulose powders[10] and by GRAHAM's[1] experiments with glucose and 15 amino acids. SHORE AND PARDEE[6] increased the intensity of the fluorescence of amino acids spots by treating the filter paper with a xylose solution before placing on it the amino acid samples. KOFRANYI[2] obtained excellent results in increasing the intensity of the amino acid fluorescence in u.v. light by means of another sensitizing

TABLE III

EFFECT OF HEATING TIME ON THE SENSITIVITY OF THE FLUORESCENCE TEST

	Amino acid	μg		
		2 min	*5 min*	*10 min*
1	Alanine	n	0.37	0.26
2	β-Alanine	0.52	0.26	0.26
3	α-Amino-*n*-butyric acid	n	0.52	0.26
4	Arginine	n	0.32	0.26
5	Asparagine	n	n	0.32
6	Aspartic acid	n	n	0.32
7	Glutamine	n	0.52	0.32
8	Glutamic acid	0.65	0.65	0.32
9	Glycine	n	0.32	0.26
10	Histidine	n	n	0.32
11	Hydroxyproline	n	1.7	1.0
12	Isoleucine	n	0.65	0.32
13	Leucine	0.65	0.65	0.32
14	Lysine	0.65	0.26	0.13
15	Methionine	n	n	0.32
16	Norleucine	0.65	0.65	0.32
17	Norvaline	0.52	0.32	0.26
18	Ornithine	0.52	0.32	0.26
19	Phenylalanine	n	0.32	0.32
20	Proline	3.4	2.6	1.8
21	Serine	0.52	0.32	0.26
22	Taurine	n	0.32	0.26
23	Threonine	n	0.52	0.32
24	Tryptophan	0.26	0.13	0.032
25	Valine	0.52	0.32	0.32

n = lack of fluorescence.

TABLE IV

SENSITIVITY OF THE U.V. TEST APPLIED TO CHROMATOGRAMS DEVELOPED IN THE SYSTEM PHENOL–WATER

	Amino acid	S_A μg	S_{ph} μg	P_{ph} mm²	$S_{R'}$ μg	K_{ph}
1	Alanine	0.26	0.65	70	0.46	1.7
2	β-Alanine	0.26	0.43	140	0.16	0.6
3	α-Amino-*n*-butyric acid	0.26	0.85	110	0.38	1.4
4	Arginine	0.26	1.04	85	0.61	2.3
5	Asparagine	0.32	0.85	100	0.42	1.3
6	Aspartic acid	0.32	0.85	70	0.60	1.8
7	Glutamine	0.32	1.3	130	0.50	1.5
8	Glutamic acid	0.32	0.65	70	0.46	1.4
9	Glycine	0.26	0.65	100	0.32	1.2
10	Histidine	0.32	0.85	90	0.47	1.4
11	Hydroxyproline	1.05	12.48	270	2.8	2.4
12	Isoleucine	0.32	1.7	110	0.77	2.4
13	Leucine	0.32	1.48	110	0.67	2.0
14	Lysine	0.13	0.52	130	0.20	1.5
15	Methionine	0.32	1.3	110	0.59	1.8
16	Norleucine	0.32	1.48	80	0.92	2.8
17	Norvaline	0.26	1.48	140	0.53	2.0
18	Ornithine	0.26	0.65	60	0.54	2.0
19	Phenylalanine	0.32	1.7	100	0.85	2.6
20	Proline	1.8	15.5	250	3.1	1.7
21	Serine	0.26	0.52	120	0.21	0.8
22	Taurine	0.26	0.32	130	0.12	0.4
23	Threonine	0.32	0.85	100	0.42	1.3
24	Tryptophan	0.032	1.3	120	0.55	16.0
25	Valine	0.32	1.3	100	0.65	2.0

S_A = Specific sensitivity.
S_{ph} = Sensitivity after development.
P_{ph} = Area of the spot.
$S_{R'}$ = Reduced sensitivity after development.

$$K_{ph} = \frac{S_{R'}}{S_A}; \qquad S_{R'} = S_{ph}\frac{50.24}{P_{ph}}.$$

reagent belonging to the group of naphthoquinone compounds. He does not explain, however, the mechanism by which 1,2-naphthoquinone-4-sulphonate renders the filter paper more sensitive. In any case, a stronger contrast is produced between the amino acid spots and the remaining surface of the filter paper. VEN HORST and co-workers[9] carried out quantitative determinations of the intensity of the amino acid fluorescence in u.v. light using a photodensitometer specially adapted to this purpose.

We were interested in several questions connected with the u.v. test. One of them was the problem of differences in its sensitivity for the individual amino acids, since investigations on the amino acid composition of substances are considerably hindered by the fact that the sensitivity ranges for the different amino acids in the ninhydrin, isatin and alloxane reactions oscillate within broad limits.

The second question was the comparison between the sensitivity of the u.v. test for amino acids and the sensitivity of other tests, especially of the generally used ninhydrin reaction. It is probable that a combination of both methods will allow

TABLE V

SENSITIVITY OF THE U.V. TEST APPLIED TO CHROMATOGRAMS DEVELOPED IN THE SYSTEM PROPANOL–WATER

	Amino acid	S_A μg	S_{pr} μg	P_{pr} mm^2	$S_{R'}$ μg	K_{pr}
1	Alanine	0.26	0.37	110	0.17	0.65
2	β-Alanine	0.26	0.37	100	0.18	0.69
3	α-Amino-*n*-butyric acid	0.26	0.85	90	0.46	1.76
4	Arginine	0.26	0.65	90	0.40	1.53
5	Asparagine	0.32	0.65	100	0.32	1.0
6	Aspartic acid	0.32	0.85	90	0.46	1.42
7	Glutamine	0.32	1.04	100	0.52	1.62
8	Glutamic acid	0.32	0.65	90	0.40	1.25
9	Glycine	0.26	0.26	90	0.14	0.53
10	Histidine	0.32	0.65	70	0.52	1.62
11	Hydroxyproline	1.05	12.48	240	2.3	2.1
12	Isoleucine	0.32	1.48	70	1.06	3.31
13	Leucine	0.32	1.7	70	1.21	3.36
14	Lysine	0.13	0.13	75	0.08	0.61
15	Methionine	0.32	1.3	70	0.93	2.90
16	Norleucine	0.32	1.48	80	0.92	2.87
17	Norvaline	0.26	1.3	90	0.72	2.69
18	Ornithine	0.26	0.52	130	0.20	0.76
19	Phenylalanine	0.32	1.3	75	0.87	2.71
20	Proline	1.8	13.0	250	2.8	1.4
21	Serine	0.26	0.85	110	0.38	1.18
22	Taurine	0.26	0.52	70	0.37	1.15
23	Threonine	0.32	1.3	110	0.67	2.09
24	Tryptophan	0.032	0.65	125	0.26	8.12
25	Valine	0.32	1.3	70	0.93	2.90

S_A = Specific sensitivity.
S_{pr} = Sensitivity after development.
P_{pr} = Area of the spot.
$S_{R'}$ = Reduced sensitivity after development.

$$K_{pr} = \frac{S_{R'}}{S_A}; \qquad S_{R'} = S_{pr}\frac{50.24}{P_{pr}}.$$

detection and identification in chromatograms of quite small amounts of those amino acids whose sensitivities in both tests are considerably different.

The third and last question concerned the prospects of finding the optimal experimental conditions for the u.v. test which might make possible the detection and identification of amino acids occurring in various concentrations in biological fluids.

The present investigations were based on the method used for determination of sensitivity in the ninhydrin, isatin and alloxane reactions[3,4,7]. The smallest amount of an amino acid which under standard experimental conditions produces fluorescence of the whole standard area of the spot was considered as the specific sensitivity. As Table I and Fig. 1 show, the sensitized u.v. test best meets our requirements. The specific sensitivities range here between 0.03 and 0.13 μg, the ordinary u.v. test shows a broader range: 0.03 to 1.8 μg. In the ninhydrin test the specific sensitivities range between 0.02 and 0.52 μg, and in the isatin reaction between 0.03 and 1.0 μg. Detailed

TABLE VI

DETECTION OF AMINO ACIDS IN URINE AND SERUM SAMPLES BY MEANS OF THE U.V. TEST AND THE NINHYDRIN TEST

	Amino acid	*25 µl urine*					*50 µl urine*					*100 µl urine*					*250 µl serum*				
		u.v.	N_s	*u.v.*$_s$	N_{ss}	*N*	*u.v.*	N_s	*u.v.*$_s$	N_{ss}	*N*	*u.v.*	N_s	*u.v.*$_s$	N_{ss}	*N*	*u.v.*	N_s	*u.v.*$_s$	N_{ss}	*N*
1	Glycine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Serine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Histidine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	Alanine	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Threonine	—	+	—	—	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Lysine	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Glutamine	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	Tyrosine	—	—	+	—	+	—	+	+	—	+	+	+	+	+	+	+	+	+	+	+
9	Valine	—	—	+	—	—	—	+	+	—	+	—	+	+	—	+	+	+	+	+	+
10	Asparagine	—	—	—	—	—	+	—	+	—	+	+	+	+	+	+	+	+	+	+	+
11	Aspartic acid	—	—	—	—	—	—	—	—	—	+	—	—	—	—	+	+	+	+	+	+
12	Glutamic acid	—	+	+	—	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+
13	Lysine	—	—	—	—	+	—	—	+	—	+	—	—	+	+	+	+	+	+	—	—
14	Leucine	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	+	+	+	+	+
15	α-Amino-*n*-butyric acid	—	—	—	—	—	—	—	—	—	—	+	—	+	+	+	—	—	+	—	—
16	β-Amino-isobutyric acid	—	—	—	—	—	—	—	—	—	—	—	+	+	—	+	—	—	—	—	—
17	Yellow spot	—	—	—	—	—	—	+	+	—	+	—	+	+	+	+	—	—	—	—	—
18	Spot a	—	—	—	—	—	—	—	—	—	—	—	+	—	+	+	+	+	—	—	—
19	Spot b	—	—	—	—	+	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+
20	Total	3	8	9	5	11	7	11	13	8	15	11	16	17	15	19	16	16	16	14	14

N_s = Ninhydrin test subsequent to u.v. test.
N_{ss} = Ninhydrin test subsequent to u.v.$_s$ test.
N = Ninhydrin test alone.

studies show that there are comparatively great differences in the sensitivity of the u.v. test and of the ninhydrin and isatin reactions for the individual amino acids. For example, in the ninhydrin reaction glycine shows the greatest specific sensitivity (0.02 μg), proline and taurine the lowest (0.52 μg). In the u.v. test the highest specific sensitivity is shown by tryptophan (0.03 μg) and the lowest by proline (1.8 μg). The absence of the proper amino group in proline and hydroxyproline explains the weak fluorescence of these amino acids. In most of the amino acids we studied, the specific sensitivity of the sensitized u.v. test was nearly ten times higher than that of the ordinary u.v. test. Only a few amino acids, such as asparagine, lysine and tryptophan, were a definite exception to this (Table I).

The results of the present investigations on the optimal conditions for the u.v. test with regard to temperature and time of heating before testing the fluorescence correspond to the results obtained by VEN HORST and co-workers. The optimal temperature for heating chromatograms was found to be 110–120°, the optimum heating time 10 minutes. When the heating temperatures are lower than 50°, none of the amino acids studied produces fluorescence; only when heated to 110–120° for 10 minutes, do all amino acids fluoresce (Tables II and III).

Our previous comparative investigations on the sensitivity of the ninhydrin reaction before and after development of the chromatograms gave different results for the individual amino acids. These differences are caused by losses of amino acids during their migration in the filter paper.

The greatest losses were observed in amino acids with the highest R_F values. Factors probably contributing to these losses are: adsorption on the filter paper, decomposition of the substances during migration, chemical interaction of the amino acids and solvents, and impurities contained in the filter paper. The u.v. test also showed losses of amino acids during the development of chromatograms.

Comparison of the results expressed in the coefficients $K_{\text{phen.}}$ disclosed that the losses are the greatest in tryptophan ($K_{\text{ph.}} = 16$, $K_{\text{pr.}} = 8$), and the smallest in taurine ($K_{\text{ph.}} = 0.4$) and lysine ($K_{\text{pr.}} = 0.6$).

The identification of amino acids contained in urine and blood serum can be carried out by means of the sensitized u.v. test and the ninhydrin test applied successively.

When the u.v. and ninhydrin tests were applied successively in order to detect amino acids in chromatograms of urine and blood serum (Table VI), the results obtained in both tests were more or less identical, providing that the volumes of urine placed on the filter paper were not too small (not below 100 μl).

The ordinary u.v. test is undoubtedly less sensitive for the majority of amino acids than the ninhydrin reaction; that is why in small volumes of urine (25 μl) the u.v. test is unable to detect all those amino acids that can be detected by means of the ninhydrin reaction.

The sensitized u.v. test, on the other hand, equals the ninhydrin reaction as far as sensitivity is concerned, but it involves greater losses of amino acids. The losses are especially marked when small volumes of urine (25 μl) are placed on the filter

paper. Successive application of both tests, one immediately after the other, though very convenient, may therefore be made use of only for qualitative and approximately quantitative estimation of amino acids.

The numerical data presented in the tables are by no means constant characteristic values; neither can they be exactly reproduced in further experiments. Even when all other experimental conditions were the same, the use of two lamps of the same type gave different results. The age of the filter is probably also of importance.

Thus the values tabulated in this article should be regarded as examples to be used for comparative purposes.

SUMMARY

Comparative investigations were carried out on the sensitivity of the ordinary and sensitized u.v. test for amino acids. To sensitize the test the filter paper was impregnated with 1,2-naphthoquinone-4-sulphonate. In the experiments, the u.v. lamp with Wood's filter (3500 Å) and the "Chromatolit" lamp (2537 Å) were used. For most of the amino acids investigated the sensitivity of the ordinary u.v. test was lower than that of the ninhydrin reaction. The sensitivity of the sensitized u.v. test proved to be ten times higher than that of the ordinary u.v. test for the majority of amino acids. Only in the case of asparagine, lysine and tryptophan was the increase of sensitivity slight. As with the ninhydrin and isatin reactions, the development of the chromatograms has an unfavourable influence on the sensitivity of the u.v. test. Greatest losses were observed in amino acids with high R_F coefficients, such as tryptophan, leucine, methionine and valine.

The optimal experimental conditions for the u.v. test were determined; chromatograms should be heated to 110–120°, and the time of heating should be 10 minutes.

The identification of amino acids contained in urine and blood can be carried out by means of the common or sensitized u.v. tests and the ninhydrin test applied successively, providing that the volumes of urine applied are not too small (about 100 μl).

REFERENCES

1 W. D. GRAHAM, P. Y. HSU AND J. MCGINNIS, *Science*, 110 (1949) 217.
2 E. KOFRANYI, *Z. physiol. Chem.*, 299 (1955) 129.
3 J. OPIEŃSKA-BLAUTH, H. KOWALSKA AND M. PIETRUSIEWICZ, *Ann. Univ. Mariae Curie-Sklodowska, Lublin-Polonia, Sec. D.*, 11 (1956) 175.
4 J. OPIEŃSKA-BLAUTH, H. KOWALSKA AND M. PIETRUSIEWICZ, *Chem. Anal. (Warsaw)*, 2 (1957) 266.
5 D. M. P. PHILLIPS, *Nature*, 161 (1948) 53.
6 V. G. SHORE AND A. B. PARDEE, *Anal. Chem.*, 28 (1956) 1479.
7 A. SEIFER AND J. ORESKES, *Anal. Chem.*, 28 (1956) 501.
8 R. W. STORHERR, *Anal. Chem.*, 31 (1959) 269.
9 H. VEN HORST, H. TANG AND V. JURKOVICH, *Anal. Chem.*, 31 (1959) 135.
10 A. J. WOIWOD, *Biochem. J.*, 45 (1949) 412.
11 A. J. WOIWOD, *Nature*, 166 (1950) 272.